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Notes on the Development of the Crab-Eating Frog, *Rana cancrivora*

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ABSTRACT—Fertilized eggs were obtained from a pair of crab-eating frogs collected in a mangrove swamp in Thailand at the end of April. The tadpoles grew well when both parents and eggs were maintained in 10% seawater, although eggs from a pair of frogs kept in 50% seawater did not develop in 10% seawater. Clutch size was about 1800. Each egg was 1.2–1.3 mm in diameter. Embryonic development was fairly rapid. Hatching took place 27 hr after fertilization at 24.5–26.0°C. Individual variation in the progress of embryonic and larval development was large. In the most rapidly growing tadpoles, metamorphosis took place 44 days after spawning in 10% seawater. On the other hand, at higher salinities (40–100% seawater) development tended to be delayed and tadpoles remained between stages V and XV (Taylor-Kollros stages) 55 days after fertilization.

INTRODUCTION

The tadpole of the crab-eating frog, *Rana cancrivora,* is the only amphibian larva which lives naturally in brackish water [1, 2]. However, it is not clear what mechanisms make this possible. It is, therefore, important to raise tadpoles of this species in the laboratory as a first step toward elucidating the physiological mechanisms of salt water adaptation. Alcala [1] described the development of this tadpole raised from eggs collected in the field with two tables and two figures. However, he did not show the correct timetable subsequent to fertilization. Later, Gordon and Tucker [2] reported failure to raise artificially fertilized eggs of this frog in various dilutions of seawater.

In the present study, spawning was induced by injection of pituitary homogenates and development was observed carefully under laboratory conditions. The embryonic stages were judged according to the developmental stages for *R. pipiens* described by Witschi [3] and the subsequent larval stages were those described by Taylor and Kollros [4].

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MATERIALS AND METHODS

Adult males and females of the crab-eating frog, *Rana cancrivora,* were captured around prawn culture-ponds (salinity 33‰) located in a mangrove swamp at Ang-Sila near Bangkok, Thailand, in late April 1987. They were shipped by air to the laboratory in Niigata, Japan and maintained in 10–50% seawater (3.5–18‰) at 25.0–26.0°C. Body weight was 20–60 g for both sexes. On June 15 1987, two pairs of the adult frogs kept in 10% seawater were injected with pituitary homogenate (one pituitary gland per frog) of *R. brevipoda porosa* and kept in a plastic tank containing 10% seawater. A pair of frogs was found to be clasping the following day and repeat injections were given to them. At 6 hr after the 2nd injection, spawning took place and the eggs were fertilized simultaneously by the male. The fertilized eggs were maintained in 10% seawater at 24.5–26.0°C. Some of the eggs were kept in small dishes for detailed observations on development. When the embryos reached stage 23, they were divided into small groups (5–20 tadpoles). All observations on developmental stage were made using a binocular dissecting microscope. Tadpoles were anesthetized by means of ice and body length was measured. Pictures were taken through the dissecting

microscope. After the tadpoles had started to swim, they were transferred into tall-skirted dishes kept at 28.0°C. The tadpoles were fed on freshly bioled spinach twice a week, and the water (10% seawater) was changed every day.

Acclimation to various dilutions of seawater

Eggs were transferred into aged tap-water, 10% and 20% seawater. Tadpoles of stages 21-XV were acclimated directly to various dilutions of seawater (tap-water to 100% seawater). Tadpoles of stages 24-XV were also acclimated in steps with 10–20% changes of salinity every 2–7 days.

OBSERVATIONS

Breeding behavior and spawning

As noted above, injection with the pituitary homogenates induced breeding behavior: the clasping is axillary and the male having pigmented vocal sacs pushed rhythmically the female's side and called. This breeding behavior was also observed in mature frogs kept in 50% seawater upon injection of pituitary homogenate and transfer to 10% seawater. Six hr later, eggs were laid. Eggs from parents that had been kept in 10% seawater developed rapidly. On the other hand, eggs from parents that had been kept in 50% seawater did not develop.

Eggs and early developmental stages

Fertilized eggs formed egg masses of 6 to several hundreds of eggs and floated on the surface of the water. Eggs were about 1.2–1.3 mm in diameter and were encapsulated within two transparent layers, the outer layer being very sticky. Clutch size was about 1800. The color of the eggs was brown in the animal hemisphere and yellowish-white in the vegetal one. Embryonic development proceeded fairly rapidly and hatching was observed 27 hr after fertilization. Before hatching, embryos actively moved their tails within the capsule (Fig. 1). After hatching, embryos lay on the bottom of the tank. Thirty hr after fertilization, the external gills were completely developed and the tadpoles swam around. Thereafter, the right external gills began to be covered with the opercular fold, and 96 hr after fertilization both sides of the operculum were completed with a respiratory pore in the left side. The tadpoles at stage 25 were ventro-dorsally compressed. The dorsal and ventral fins were relatively wide. The abdomen was convex and the cloaca opened to the right side. These observations are summarized in Table 1 and Figure 2A-Q.

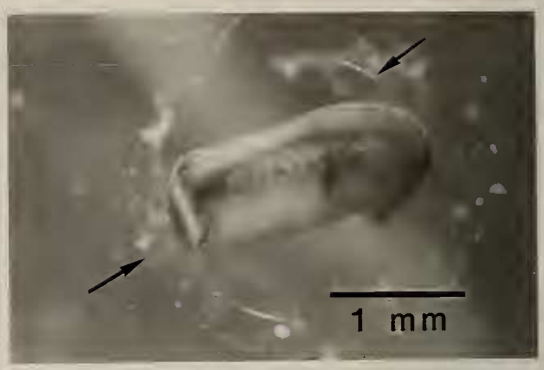

Fig. 1. Stage 20. An embryo moving its tail actively just before hatching. Arrows indicate two layers of the capsule.

Larval development (*limb bud stage-juvenile*)

Fourteen days after fertilization, a pair of hindlimb buds emerged. In tadpoles at stage IV, the tooth row was fully developed with the dental formula, $\frac{1:1+1}{3}$ (Fig. 3).

The tadpoles usually stayed on the bottom of the dish. Before stage I, the skin of the tadpoles was translucent, the color of the back brown, and melanophores were deposited on the dermis of the abdomen. Tadpoles after stage I were yellowish-

Fig. 2. A, Stage 1; B, Stage 5; C, Stage 6; D, Stage 7; E, Stage 8; F, Stage 13; G, Stage 14; H, Stage 15; I, Stage 16; J, Stage 18; K, Stage 19; L, Stage 20; M, Stage 21; N, Stage 22; O, Stage 23; P, Stage 24; Q, Stage 25; R, Stage IV; S, Stage XV; T, Stage XX; U, Stage XXII, V, Stage XXIV; W, Stage XXV.
Scale bars indicate 1 mm (A-Q), and 1 cm (R-W).
Arrow indicates deposit of guanophores on the abdominal skin in tadpole of stage XV.

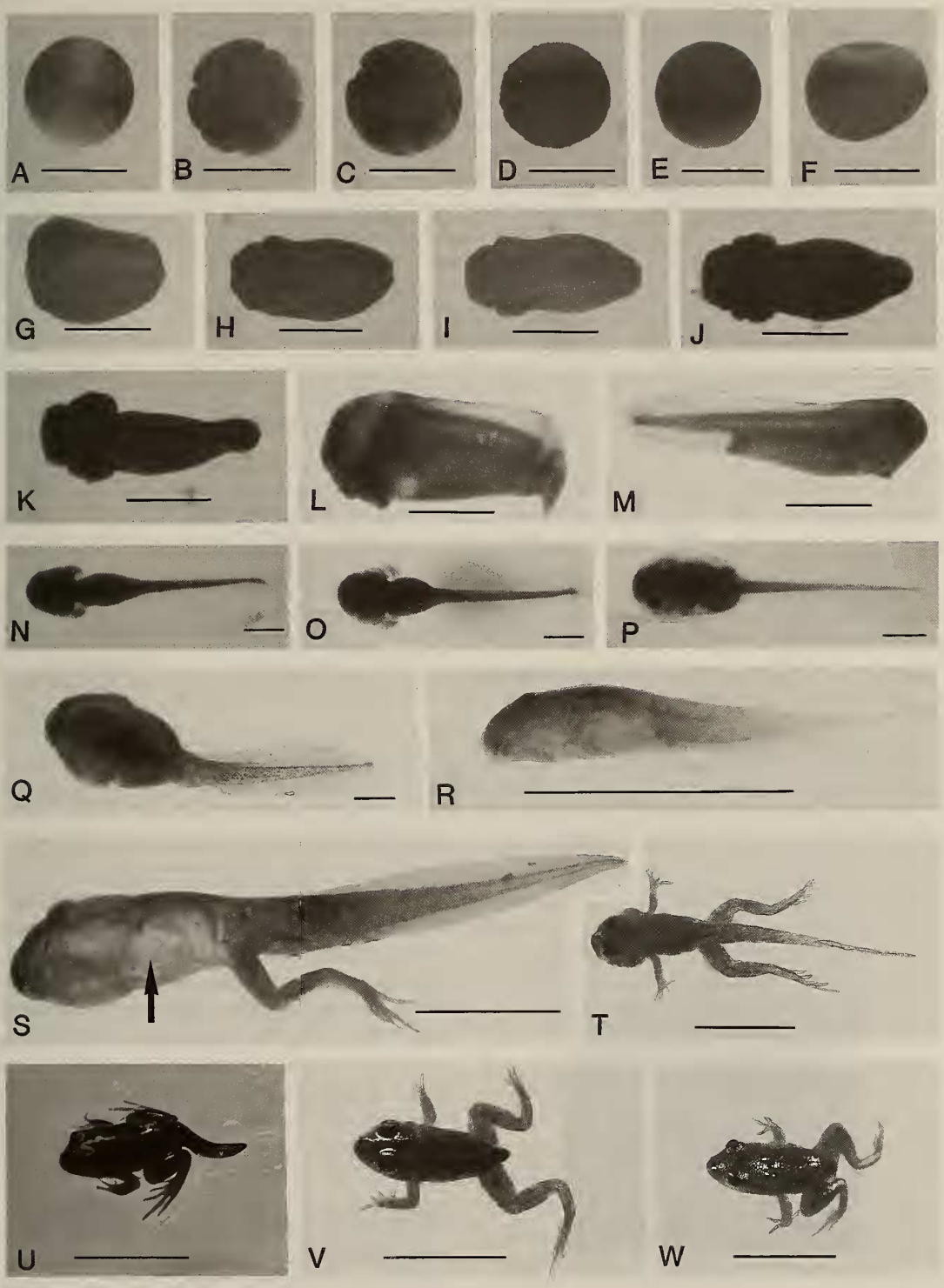
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Table 1. Early larval development of *Rana cancrivora* in 10% seawater (water temperature 24.5–26°C)

Stage	Time after fertilization (hr:min)	Notes
1	0:05	Egg is encapsulated by two layers. Diameter of egg is 1.2–1.3 mm (Fig. 2A).
4	1:20	Period of cleavage (Fig. 2B-D).
6	3:20	
8	4:30	Blastula stage (Fig. 2E).
11	7:30	Period of gastrulation.
12	8:30	Primitive streak stage.
13	10:30	Embryo elongated. Flattened on dorsal surface of embryo (Fig. 2F).
14	11:30	Blastopore closed (Fig. 2G).
15	12:30	Neural plate stage (Fig. 2H).
16	14:00	Neural tube stage (Fig. 2I).
17	15:30	Tail bud stage. *Total length 1.8 mm.
18	16:30	Tail bud elongated (Fig. 2J). Oral sucker distinct. Total length 1.9 mm.
19	18:00	The 1st and 2nd external gill buds become visible (Fig. 2K). Total length 2.05 mm.
20	25:00	Tail of embryo is curved (Fig. 2L).
21	27:00	Spontaneous hatching (Fig. 2M). Lavae lie on the bottom. Total length 3.4–4.4 mm.
22	30:00	Larvae bigin to swim. Body is asymmetrical (Fig. 2N). Total length 4.0–4.2 mm.
23	51:00	Abdomen becomes round (Fig. 2O). Total length 4.0–5.5 mm.
24	62:00	Right external gills are covered by opercular fold. Total length 6.0–6.2 mm.
25	96:00	Left external gills are covered (Fig. 2P, Q). Total length 6.2–6.5 mm.

* Total length is defined as the length from tip of snout to tip of tail.

Fig. 3. Oral part of tadpole at stage X, dental formula being $\frac{1:1+1}{3}$.

gray in color on the back and silver on the abdomen due to guanophore deposition. Development proceeded fairly rapidly. It took 44 days after fertilization to begin metamorphosis in the most rapidly growing tadpole. There were large individual differences in the progress of larval development, but no cannibalism was observed. The froglets at the metamorphic climax (stage XXIII) could not climb the glass wall of the dish. These observations are summarized in Table 2 and Figure 2R-W.

Acclimation to various dilutions of seawater

Fertilized eggs developed well in tap-water, and 10% and 20% seawater. When hatchlings (stage 22) were transferred directly from 10% seawater to

TABLE 2. Larval development of *Rana cancrivora* in 10% seawater (water temperature 28°C)

Stage	Time after fertilization* (days)	Total length** (mm)	Notes
I	15***	6.6–10.0***	Limb buds become visible.
III	33	6.6–12.5	Length of limb bud equal to its diameter.
IV	20	17.0	Horny teeth fully developed (Fig. 2R).
V	24	21.1	Pigmentation evident on abdomen.
VI	27–43	26.0–29.0	Limb buds paddle-shaped.
X	27–52	31.1–32.0	Five toes distinct.
XII	44	28.0	
XIII	50	32.0	
XIV	44	30.0–35.0	Toe pads appear.
XV	30–50	33.0–45.0	Hindlimbs elongate (Fig. 2S).
XVIII	44	37.0–42.0	Cloacal tail-piece disappears.
XIX	39	33.0–40.0	Skin windows become clear.
XX	36		Forelimbs appear (Fig. 2T).
XXI	39		Tail fins absorbed.
XXII	50	26.0–32.0	Tail shorter than hindlimbs (Fig. 2U).
XXIII	42–46	19.0	
XXIV	43	13.0	Stub of tail remains (Fig. 2V).
XXV	44		Metamorphosis accomplished (Fig. 2W).

* Individual variation in the progress of development was large.
** Total length is defined as the length from tip of snout to tip of tail and to vent in tadpole and frog, respectively.
*** Number of animals used for measurements was 1–5.

various dilutions of seawater (tap-water to 100%), they became well acclimated to environments up to 40% seawater. However, tadpoles transferred to 50% seawater or higher concentrations could not acclimated and all died within 8 hr. When environmental salinity was increased stepwise, tadpoles (stages I-XV) were able to adapt to higher salinities (tap-water to 100% seawater). Metamorphosis took place in tadpoles kept in tap-water and 10% seawater. On the other hand, at 55 days after fertilization, tadpoles kept in 40–100% seawater still remained between stages V and XV, without any indication of metamorphosis.

DISCUSSION

This is probably the first report on fertilized eggs of *R. cancrivora* being obtained by artificial induction of spawning and on young frogs being raised in the laboratory. The characteristics, including the size and time of development, of eggs and tadpoles observed in the present study were fairly consistent with those reported by Alcala [1]. Therefore, the present observations seem to reflect the normal breeding behavior and development of this species in the field. The developmental process of this species is similar to that of *R. pipiens*, except that development proceeds very rapidly. In the present study, metamorphosis took place in the most rapidly growing tadpoles 44 days after spawning at about 28°C. On the other hand, Taylor and Kollros [4] reported that *R. pipiens* larvae metamorphosed at an age of over 90 days at room temperature (about 20°C).

Gordon and Tucker [2] reported that no development was observed when eggs from frogs kept in 60% seawater were artificially fertilized in 20% seawater. However, the first few cleavages occurred when eggs from adults kept in 20% seawater were artificially fertilized and placed in either fresh water or 20% seawater. They [2] also suggested from their field data that spawning might

occur only during and soon after heavy rains when the salinity of the spawning pools becomes low. In the present study, frogs kept in 50% seawater showed breeding behavior, but eggs were undeveloped even when removed to 10% seawater after fertilization. These results, therefore, suggest that it is necessary for frogs and eggs to be kept in hypoosmotic media in order for them to develop.

In the tolerance experiments, although the tadpoles at developmental stages III-XIX were able to survive well at all salinities from fresh water to full-strength seawater (32‰), eggs and tadpoles before stage 25 could not stay in high salinity [2, 5]. Early development (stages 1–25) proceeded rapidly until 4 days after fertilization, and it then took about 10 days until the appearance of limb buds (stage I). During the period between stage 25 and stage I, the skin grew to be thick. This morphological change may be one of the factors for salt tolerance of tadpoles at these stages. Gordon and Tucker [2] observed that metamorphosis is hindered by salinities higher than 20% seawater, and suggested from the field data that metamorphosis is delayed as long as the pond salinity remains high. The present observations are consistent with their suggestion. Metamorphosis took place in tadpoles kept in tap-water and 10% seawater and development of tadpoles kept in higher salinities (40–100% seawater) was delayed. These results may suggest that a low-salinity environment is necessary for the induction of both metamorphosis and spawning.

According to the previous observations [1, 2] and the present study, it can be speculated that the following may occur in nature. After heavy rainfall during the rainy season, spawning occurs and early embryonic development proceeds rapidly in low-salinity and high-temperature water. Then, when the tadpoles acquire salinity tolerance they can survive in higher-salinity water. Thereafter, the development of tadpoles proceeds further and metamorphosis is accomplished when water salinity becomes low after the next heavy rainfall. The rapid advance of development in this species must therefore be favored by an environment where the salinity changes markedly.

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